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Emotion Regulation Training for Treating Warfighters with Combat-Related PTSD
Using Real-Time fMRI and EEG-Assisted Neurofeedback

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14. ABSTRACT PTSD is a chronic and disabling condition. Neurocircuitry-based models of PTSD emphasize dysregulation of the amygdala, which is involved in the regulation of PTSD-relevant emotions. We are utilizing real-time functional magnetic resonance imaging neurofeedback (rtfMRI-nf) training with concurrent electroencephalography (EEG) recordings to directly target and modulate the emotion regulation neurocircuit. By using multimodal data, we can determine which EEG signals/leads or their combination specifically predicts or correlates with clinical improvement associated with the rtfMRI-nf training. Difficult recruitment is the main reason behind the delayed study schedule (currently 2 nd year no cost extension). During year 4 of the project we have improved our recruitment, finished rtfMRI-nf and EEG data collection, and started EEG-only data collection. Data analysis indicates amygdala training with concurrent EEG recordings in a combat-related PTSD population is feasible, tolerated well and this procedure resulted in improvements in PTSD symptoms. We identified the variations in frontal upper alpha EEG asymmetry (FEA) during the rtfMRI-nf amygdala training as a promising measure of PTSD severity and treatment response. We are employing this measure together with our already developed stand-alone EEG-only neurofeedback training protocol to evaluate FEA EEG-nf training feasibility in combat-related PTSD.					
15. SUBJECT TERMS PTSD; amygdala; fMRI; EEG; real-time fMRI neurofeedback; simultaneous EEG-fMRI; emotion regulation					
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Table of Contents

	<u>Page</u>
1. Introduction	4
2. Keywords	4
3. Overall Project Summary	5
4. Key Research Accomplishments	28
5. Conclusion	28
6. Publications, Abstracts, and Presentations	30
7. Inventions, Patents and Licenses	32
8. Reportable Outcomes	32
9. Other Achievements	33
10. References	34
11. Appendices	36

1. INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Our *main objective* is to determine whether rtfMRI- and rtEEG-assisted neurofeedback emotion regulation training protocols can reduce the symptoms of combat-related post-traumatic stress disorder (PTSD), a chronic and disabling psychiatric condition. Individuals with PTSD suffer from the dysregulation of several types of emotion, including fear, anxiety, anger, and depression [1–4]. Neurocircuit models of PTSD emphasize the role of the amygdala and its reciprocal interactions with the ventromedial prefrontal cortex (vmPFC) [5–9]. To advance understanding of the treatment of combat-related PTSD, the current state-of-the-art research aims to test ways to modulate the functions of the emotion circuit implicated in PTSD. We utilize the recent advances in real-time functional magnetic resonance imaging neurofeedback (rtfMRI-nf) to directly target and modulate amygdala activity [10–11]. This technique measures neuronal activity with sufficiently high temporal resolution that information from the amygdala is immediately available to form a feedback loop. In parallel with rtfMRI-nf, we obtain simultaneous measurement of electroencephalography (EEG) signals, which directly reflect brain activity in the cerebral cortex [12]. By using the multimodal imaging data we can determine which EEG signals/leads or their combination specifically predict or correlate with clinical improvement that has been associated with the rtfMRI-nf training [11,13–16]. This knowledge will enable us to establish a translational path toward the development of stand-alone real-time EEG neurofeedback (rtEEG-nf) training for emotion regulation, which can facilitate the widespread implementation of the treatment approach due to the high portability and relatively low cost of EEG systems.

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

Combat-related PTSD, fMRI, EEG, emotions, amygdala, neurofeedback

- 3. OVERALL PROJECT SUMMARY:** Summarize the progress during appropriate reporting period (single annual or comprehensive final). This section of the report shall be in direct alignment with respect to each task outlined in the approved SOW in a summary of Current Objectives, and a summary of Results, Progress and Accomplishments with Discussion. Key methodology used during the reporting period, including a description of any changes to originally proposed methods, shall be summarized. Data supporting research conclusions, in the form of figures and/or tables, shall be embedded in the text, appended, or referenced to appended manuscripts. Actual or anticipated problems or delays and actions or plans to resolve them shall be included. Additionally, any changes in approach and reasons for these changes shall be reported. **Any change that is substantially different from the original approved SOW (e.g., new or modified tasks, objectives, experiments, etc.) requires review by the Grants Officer's Representative and final approval by USAMRAA Grants Officer through an award modification prior to initiating any changes.**

Difficult and challenging recruitment is the main reason behind the study delay schedule by approximately two years. We therefore requested on August 2, 2016 a second twelve-month, no-cost extension (beyond the original project completion date of Sep 29, 2015), which was approved. During the fourth year of the project period we have completed Phase 1 of the study (i.e., real-time fMRI neurofeedback [rtfMRI-nf] and EEG data collection) targeting Aim #1: *Establish rtfMRI-nf training feasibility with concurrent EEG recordings in a combat-related PTSD population.* We have already met and exceeded project Milestone #2: fMRI/EEG data collection of 8 subjects per group (control: veterans with no PTSD; neurofeedback, sham: veterans with PTSD). Preliminary data analysis indicates (as described below) rtfMRI-nf amygdala training with concurrent EEG recordings in a combat-related PTSD population is feasible. In parallel we have also developed the rtfMRI-nf and rtEEG-nf software (Milestone #3) for the purpose of Aim #2: *Develop a stand-alone rtEEG neurofeedback training protocol for PTSD.* We identified the variations in frontal upper alpha EEG asymmetry during the rtfMRI-nf amygdala training as a promising measure of PTSD severity and treatment response. This EEG signal feature is suitable for developing a stand-alone EEG neurofeedback training protocol (Milestone #4). We have finished preparation and begun data collection for Phase 3 of the project and Aim #3: *EEG-only neurofeedback training feasibility in combat-related PTSD.* The Phase 3 subject visit schedule is shown in Figure 1 below.

Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9 (optional)
Screening doctor (may be split into 2 visits)	Consent/baseline assessment (may be combined with visit 1)	Baseline scans	Neurofeedback #1	Neurofeedback #2 (5-9 days after visit 4)	Neurofeedback #3 (5-9 days after visit 5)	Follow-up scans (5-9 days after visit 6)	Follow-up assessments (can be combined with visit 7)	Post-sham neurofeedback
SCID screening visit Doctor visit	Consent CAPS Script development	HAM-D MADRS HAM-A	HAM-D MADRS HAM-A	HAM-D MADRS HAM-A	HAM-D MADRS HAM-A	HAM-D MADRS HAM-A	CAPS	HAM-D MADRS HAM-A
	TAS ECS BIS/BAS Word selection WASI	PCL-M SHAPS BDI POMS VAS	PCL-M SHAPS BDI POMS VAS	PCL-M SHAPS BDI POMS VAS	PCL-M SHAPS BDI POMS VAS	PCL-M SHAPS BDI POMS VAS		PCL-M SHAPS BDI POMS VAS
		EEG prep Resting state Behavioral tasks • ecStroop • SDIP • AAT	Neurofeedback education EEG prep Neurofeedback POMS VAS Post-NF Questionnaire	EEG prep Neurofeedback POMS VAS Post-NF Questionnaire	EEG prep Neurofeedback POMS VAS Post-NF Questionnaire	EEG prep Resting state Behavioral tasks • ecStroop • SDIP • AAT		EEG prep Neurofeedback POMS VAS Post-NF Questionnaire

Key
Clinician ratings
Self-assessments
EEG prep
Behavioral testing by lab

Figure 1: Phase 3 visit schedule. SCID=Structured Clinical Interview for DSM-IV, CAPS=Clinician-Administered PTSD Scale, TAS=Toronto Alexithymia Scale, ECS=Emotional Contagion Scale, BIS/BAS=Behavioral Inhibition System/Behavior Avoidance System, WASI=Wechsler Abbreviated Scale of Intelligence, HAM-D=Hamilton Rating Scale for Depression, MADRS=Montgomery–Åsberg Depression Rating Scale, HAM-A=Hamilton Rating Scale for Anxiety, PCL-M=PTSD Checklist-Military Version, SHAPS=Snaith-Hamilton Pleasure Scale, BDI=Beck Depression Inventory, POMS=Profile of Mood States, VAS=Visual Analog Scales, ecStroop=Emotional Counting Stroop, SDIP=Script-Driven Imagery Procedure, AAT=Approach-Avoidance Task

All necessary computer hardware (1 Ubuntu workstation for running stimulus, 1 Windows laptop for collecting EEG and physiological data) as well as peripheral devices (64-channel EEG caps, additional EEG power supply and amplifiers, respiration belt and GSR leads for collecting physiological data, mechanical keyboard response device for ecStroop task, joystick for AAT, headphones and volume control for the script-driven imagery procedure [SDIP], and a mobile cart for performing the experimental tasks) have been acquired. Modification of the ecStroop and SDIP tasks for phase 3 and programming of the AAT in Python have been completed. Setup of stand-alone real-time EEG data collection has been completed. A database for collecting and storing clinician ratings and self-assessment questionnaires was prepared, so phase 3 has been paperless from the start.

To continuously monitor recruitment progress, we have regular weekly meetings with recruitment staff and biweekly research meetings where current project needs, problems, method and software developments, and relevant activities are discussed with all investigators, including co-investigators Drs. Feldner (University of Arkansas) and Krueger (George Mason University), both joining via video or teleconference.

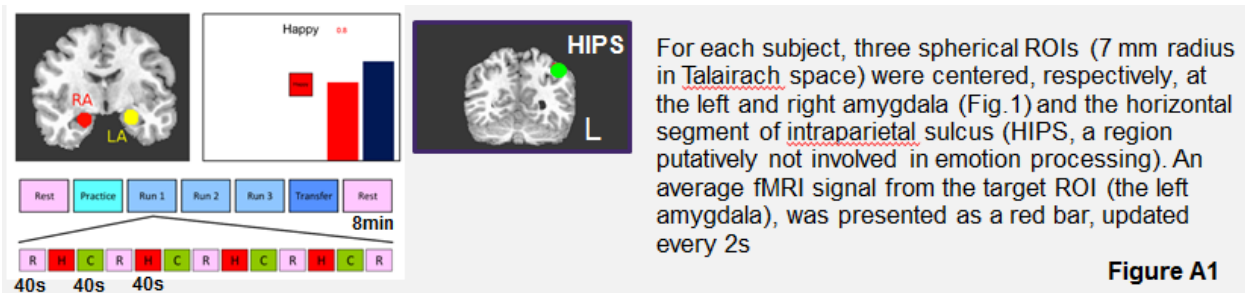
Preliminary and ongoing data analysis

Now that we have completed data collection for Phase 1 of the study, we continue to advance efficient data processing pipelines and conduct preliminary data analysis for Aim #1, which includes the following: (A) to validate whether veterans with PTSD are able to tolerate well and use rtfMRI-nf training to enhance their control of the hemodynamic response of the amygdala, and to further assess specificity of this training; (B) to evaluate possible sustained neuroplastic changes induced by the procedure; and for Aim #2; (C) to perform EEG coherence analysis to further investigate EEG correlates of the rtfMRI-nf procedure; (D) to investigate connection between frontal EEG asymmetry (identified a single EEG feature that is suitable for developed the stand-alone rtEEG-nf training protocol, Phase 2) and BOLD activity during the rtfMRI-nf training in PTSD; and (E) to conduct the EEG exploratory analysis focusing on temporally independent EEG microstates.

A) rtfMRI-nf amygdala emotional training.

Introduction: We have assessed whether veterans with PTSD and trauma exposed veteran controls are able to tolerate well and use rtfMRI-nf emotional training during happy memories recall to enhance their control of the hemodynamic amygdala response.

Methods: The updated analyses were conducted on the 25 veterans in the left amygdala (LA) feedback experimental group (EG), 11 in the control feedback (HIPS) group (CG), and 20 in the healthy (trauma control) group (TC) (Fig. A1). These analyses include results from only the first rtfMRI-nf visit.



All subjects were male, age 18–55, right-handed U.S. military combat veterans. No significant baseline differences were observed in age, PTSD symptom severity, or depression symptom severity between EG and CG. There was significant difference in PTSD and depression symptom severity, but not age, between TC and EG/CG (Table A1).

	Experimental (EG)	Control (CG)	Trauma Control (TC)
n	25	11	20
Age	31 (6)	34 (9)	34 (9)
Initial CAPS	53 (15)	57 (25)	5(5)*
Initial HRSD	15 (4)	17 (8)	3(4)*

Table A1. Demographic information for experimental (EG), control (CG), and trauma-exposed healthy (TC) groups. CAPS = Clinician Administered PTSD Scale. HRSD = 21-Item Hamilton Rating Scale for Depression. Numbers in parentheses indicate standard deviation. * indicates significant difference ($p < 0.05$) from EG/CG on an independent samples t-test

Results: ROI analysis. A GLM analysis for each of the three visits was performed to determine the training effect of the neurofeedback procedure [1]. The analyses were implemented in AFNI and SciPy. Pre-processing included our in-house developed EEG-assisted retrospective motion correction (EREMCOR) [2], white matter regression, volume registration, and slice timing correction. Standard GLM analysis was then applied separately for each of the five neurofeedback runs of each of the first visit including the following regressors: two block stimulus conditions (Happy Memories, Count), six motion parameters, and five polynomial terms. After deconvolution, percent signal change for Happy vs. Rest conditions was calculated. In preparation for the whole-brain statistical group analysis, the spatially-normalized fMRI percent signal change maps were spatially smoothed using a Gaussian kernel with full width at half maximum (FWHM) of 5 mm. One-sample *t*-tests were performed separately for each run and for each group to determine whether activation was significant.

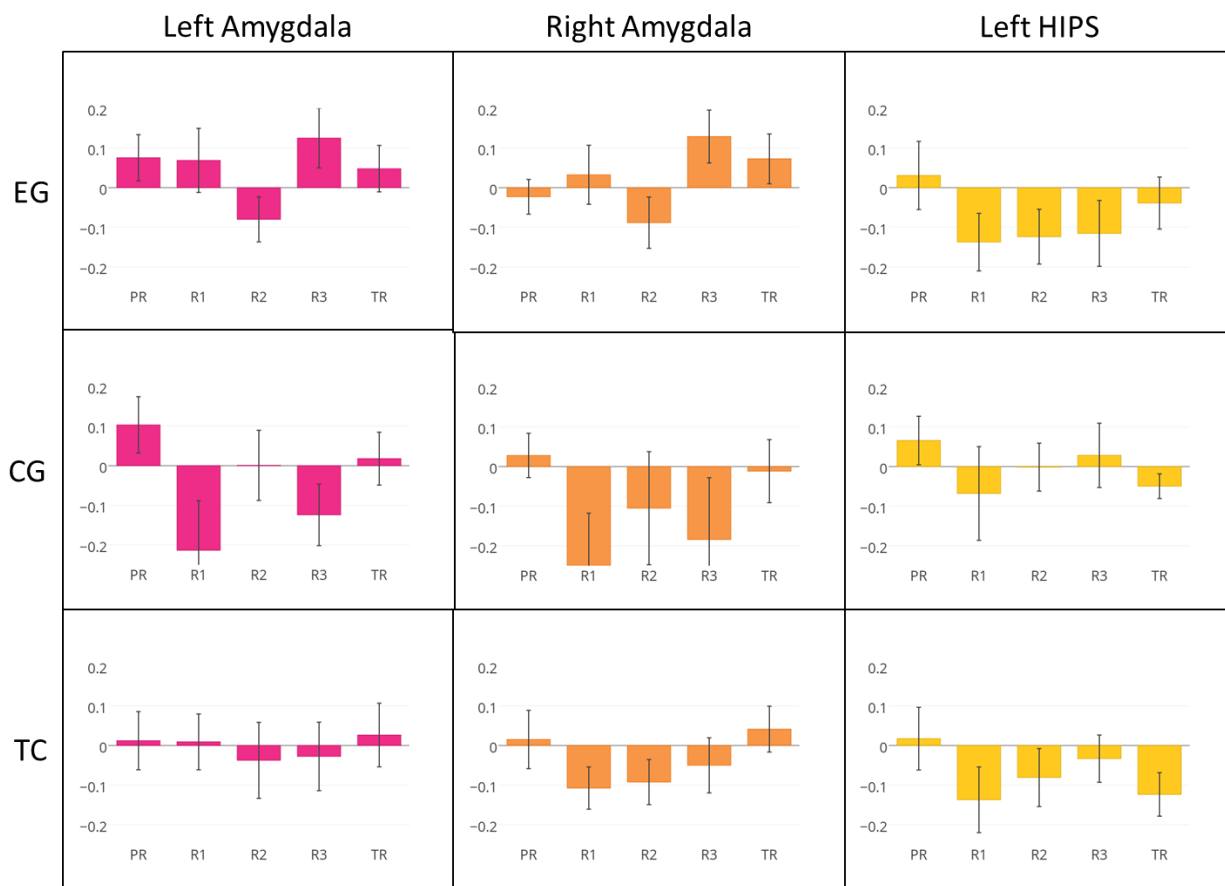


Figure A2. Mean percent signal change between Happy and Rest conditions for each of the neurofeedback runs. LA = left amygdala, RA = right amygdala, HIPS = left horizontal segment of the intraparietal sulcus, PR = practice, R1–3 = training runs 1–3, TR = transfer. EG = experimental group, CG = control group, TC = trauma-exposed healthy control group.

ROI analysis results are shown below in Figure A2. Each row of graphs represents results from one of the three subject groups. Each bar shows results for one of three ROIs: left amygdala (target ROI for the EG and TC), right amygdala (shown to explore the effects of laterality), and left HIPS (target ROI for the control group). In each graph, bar height represents mean percent signal change between Happy and Rest conditions for a single run.

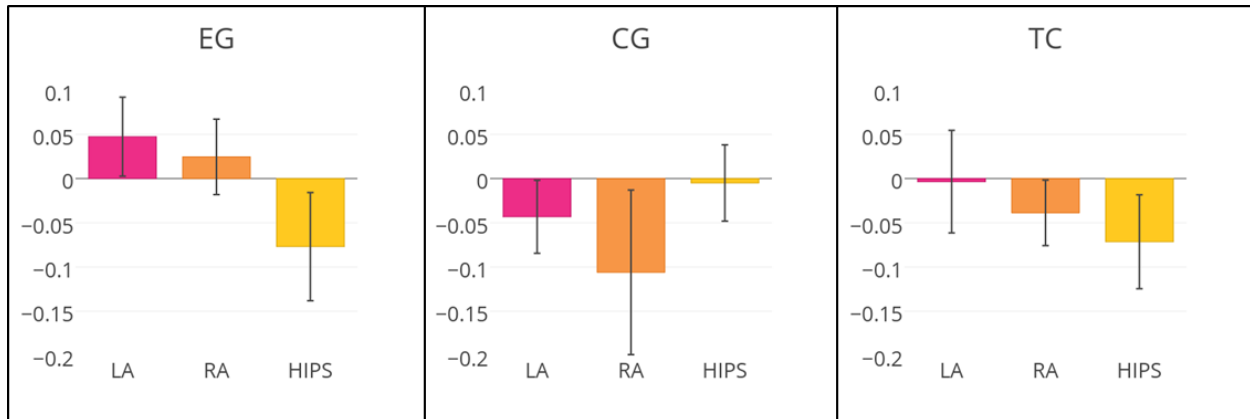


Figure A3. Mean percent signal change between Happy and Rest conditions averaged across all runs (PR, R1-R3,TR) within the first visit. Data from all three groups are shown.

Figure A3 shows for each visit a mean percent signal change between Happy and Rest conditions across all runs (PR,R1-R3,TR) within a single visit for LA, RA, and HIPS (in each bar plot: left, middle, and right columns respectively).

These results indicate that in the first visit, subjects in the experimental group were able to better elevate activity in the left amygdala during neurofeedback scans as compared to the control group. However, a large degree of variability across subjects exists, resulting in a lack of consistent statistically significant differences between the experimental and control groups. A similar activation pattern occurred in the right amygdala, though the effect for the experimental group was not as strong as in the left amygdala. Neither group was able to up regulate activity in the left HIPS.

Clinical score change results.

Clinical ratings were taken at the beginning of each visit and used to assess the effects of the neurofeedback training on PTSD and depression symptoms (Table A2). Subjects in the experimental group showed decreased PTSD and depression symptoms that were both statistically and clinically significant. Significant reduction in depression symptoms (according to the HRSD) were seen after only one neurofeedback visit and significant PTSD symptom reductions (according to the PCL-M) were observed after two neurofeedback visits.

Scale	Experimental Group			Control Group			Trauma-Exposed Healthy		
	Initial score	Mean change	t	Initial score	Mean change	t	Initial score	Mean change	t
CAPS	54	-14**	t(15)=3.8	62	-9	t(7)=1.7	4	-1	t(15)=0.5
PCL-M	46	-9**	t(18)=4.3	52	-9	t(8)=2.1	19	0	t(16)=-0.2
HRSD	17	-6**	t(19)=4.9	17	-6*	t(8)=2.7	2	+1	t(16)=-0.7
MADRS	21	-8**	t(20)=4.1	19	-6	t(8)=1.4	1	+2	t(15)=-0.7
HRSA	17	-5**	t(20)=4.4	19	-5*	t(8)=2.4	2	+1	t(16)=-0.6

Table A2. Clinical score change results for experimental, control, and trauma-exposed healthy groups. CAPS = Clinician Administered PTSD Scale (0–136). PCL-M = PTSD Checklist – Military Version (17–85). HRSD = 21-Item Hamilton Rating Scale for Depression (0–52). MADRS = Montgomery–Åsberg Depression Rating Scale (0–60). HARS = Hamilton Anxiety Rating Scale (0–56). Initial ratings taken before first neurofeedback scan. Final ratings taken at final Stroop scan (after 3rd neurofeedback scan). A significant change from pre- to post-scan ratings (paired t-test) at $p < 0.05$ marked with *, and at $p < 0.01$ marked with **.

Discussion: We have observed a large degree of individual variability across subjects during rtfMRI-nf amygdala emotional training, resulting in a lack of consistent statistically significant differences between the experimental and control groups. A similar activation pattern occurred in the right amygdala, though the effect for the experimental group was not as strong as in the left amygdala. Neither group was able to up regulate activity in the left HIPS. The rtfMRI-nf amygdala emotional training is feasible in veterans with combat related PTSD, all study participants tolerate this procedure well. Importantly only in active group, we have observed larger improvements in CAPS and PCL-M symptoms scores that were statistically and clinically significant.

References:

- [1] Zotev et al., Self-regulation of amygdala activation using real-time fMRI neurofeedback. PLoS ONE 2011 6(9), e24522.
- [2] Wong CK, et al., Automatic EEG-assisted retrospective motion correction for fMRI (aE-REMCOR) Neuroimage 2016 129:133-147

B) Whole brain exploratory analysis of altered resting-state fMRI connectivity for combat-veterans with and without PTSD and rtfMRI-nf amygdala training effect on abnormal connectivity.

Introduction: We have explored altered resting-state functional connectivity for combat veterans with and without PTSD compared to non-trauma-exposed healthy control subjects. We employed multivariate distance-based matrix regression (MDMR) analysis [1] to perform an unbiased search of whole-brain voxel-by-voxel connectivity (connectome-wide association study). We aimed to identify altered connectivity in combat veterans and then to examine changes of identified abnormalities after the rtfMRI-nf training.

Methods: *Participants.* Thirty-nine male combat veterans with PTSD and 22 male combat veterans without PTSD (veteran control, VC) participated in the resting-state fMRI scan before the rtfMRI-nf training sessions. Resting-state fMRI data of 28 age-matched non trauma-exposed healthy males participated in other study were used as control (non-trauma controls, NC). After a careful data inspection, we found that several resting-state data for PTSD and VC groups suffered from significantly large head motion compared to NC group. To address this problem we excluded four PTSD and four VC subjects from the analysis. Within veteran subjects, 27 PTSDs and 13 VCs completed all the rtfMRI-nf training sessions and the post-training resting-state scan. All VCs and 20 PTSDs received amygdala (active) neurofeedback and seven PTSDs received HIPS (sham control) neurofeedback in the rtfMRI-nf training. We had to exclude five PTSDs (4 active and 1 sham control) and two VCs resting-state data from the analysis due to severe head motion. In summary, 35 PTSD, 18 VC, and 28 NC subjects were analyzed for pre-training connectivity investigation, and 16, 6, and 11 subjects of PTSD active, PTSD sham, and VC active, respectively, were analyzed for post-training connectivity investigation.

MRI/fMRI preprocessing; Physiological noise reduction with RETROICOR/RVT, slice-timing and motion correction, nonlinear warping to the MNI template brain, spatial smoothing (4mm³ FWHM), and scaling to percent change were applied to resting-state fMRI data. Noises in signal time-course were removed by regressing out three principal components of ventricle signal, local white matter average signal (ANATICOR), motion parameters, low-frequency fluctuation (polynomial model) from the signal time course, and censoring volumes with large head motion.

MDMR analysis overview; For each subject: a) down-sampled fMRI images to 4mm³ voxels; b) extracted voxels in gray matter region; c) calculated correlation of signal time courses for each voxel to all other brain voxels and applied Fisher Z-transform to make a connectivity map for each voxel; d) calculated Euclid distance between connectivity maps of the subjects to make a distance matrix; and e) applied nonparametric MANCOVA for the distance matrix. *P* value was evaluated by a 10,000 repetition permutation test. These steps were repeated for all voxels as a seed to make a statistical parametric map.

Results: Abnormal functional connectivity in PTSD and VC before the *rtfMRI-nf* training;

Figure B1 shows the regions with significant main effect of group difference between PTSD, VC, and NC groups in MDMR analysis. These regions were used as a seed for the post-hoc analysis

that investigated which functional connectivity was significantly altered between groups in detail. Post-hoc analysis indicated that PTSD compared to NC had increased connectivity across sensory motor regions including the precentral gyrus, the intraparietal region, and the precuneus region (Fig. B2a). PTSD also showed increased connectivity between the superior temporal sulcus (STS) and

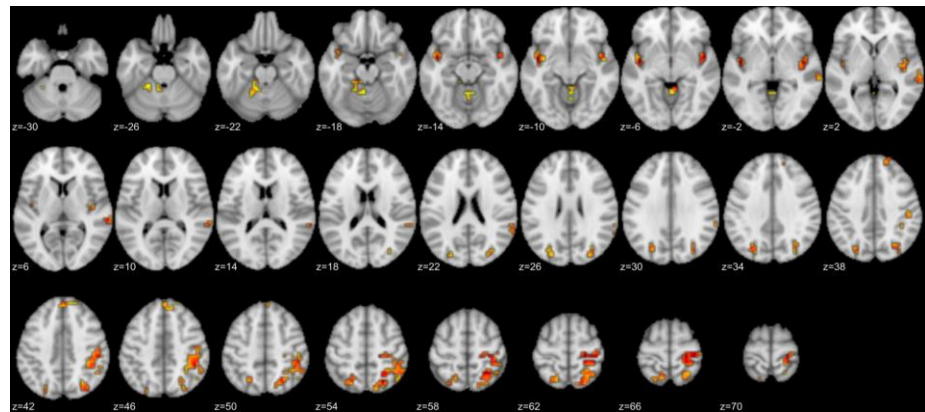


Figure B1: *F*-value map for the main effect of PTSD, VC, and NC groups in MDMR analysis ($P < 0.005$ with cluster size $P < 0.05$ by permutation test).

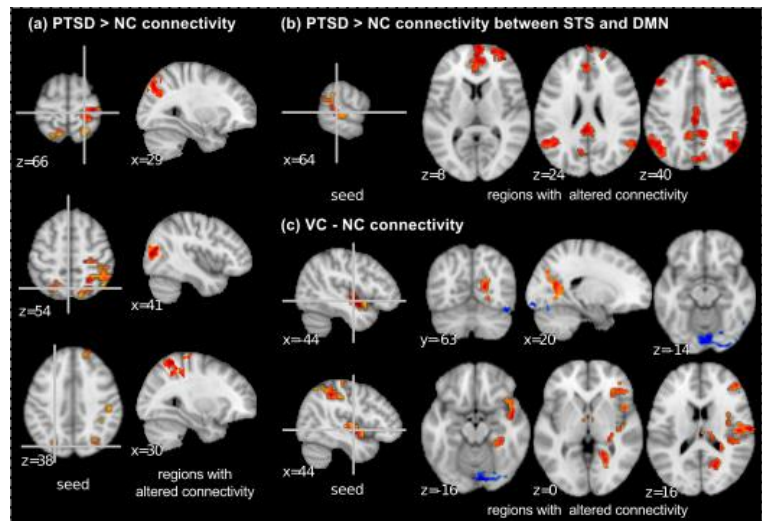


Figure B2: Altered resting-state functional connectivity between PTSD and NC (a,b), and between VC and NC (c).

the default model network (DMN) regions compared to NC (Fig. B2b). VC subjects compared to NC showed altered connectivity from the bilateral posterior insula (Fig. B2c). No significant difference was seen between PTSD and VC groups.

Effects of rtfMRI-nf training on the altered functional connectivity: All the abnormal functional connectivity identified in the previous analysis showed significant change to normalize the connectivity after the rtfMRI-nf training. Hyperconnectivity in sensory motor areas and the STS to the DMN regions for PTSD were reduced after the training (Fig. B3a). While the effect was seen for both PTSD active and PTSD sham groups, active group showed more significant reduction ($P=0.001$) than sham group ($P=0.039$). Importantly only the PTSD

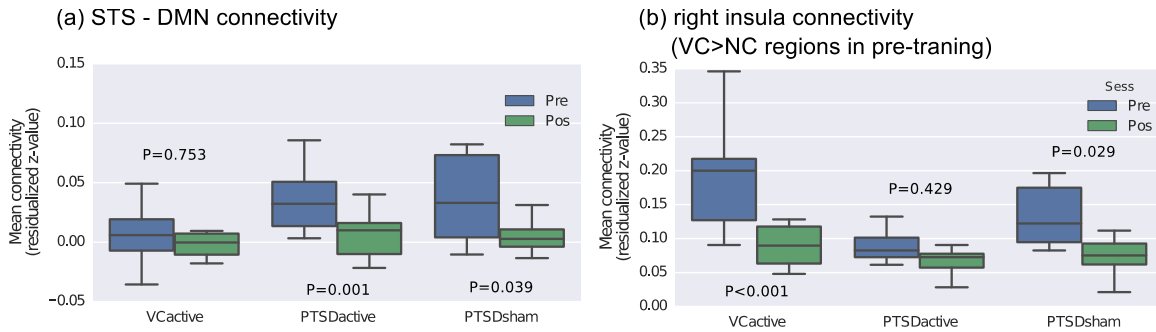


Figure B3: rtfMRI-nf training effect on abnormal resting-state functional connectivity. STS-DMN connectivity where PTSD had hyperconnectivity before the training was reduced after the training (a). Hyperconnectivity to the right insula in VC was also normalized after the training. P values indicate the significance of difference between pre- and post-session (corrected for multiple comparisons).

active group showed significant reduction of PTSD symptoms measured by PCL-M after the training ($P=0.005$). Examining subscores of PTSD symptoms revealed that training effect was significant for ‘avoidance and numbing’ symptoms ($P=0.005$) for PTSD active group. Abnormal connectivity in VC also normalized after the training (Fig. B3b) while no symptom change was observed.

Discussion: PTSD subjects had higher connectivity across sensory motor areas, which could be associated with hyperarousal symptom in PTSD. Connectivity between the right superior temporal region and the default mode network regions also increased in PTSD, which might be associated with dissociation symptom in PTSD. It has been indicated that abnormal activity in the superior temporal region is associated with dissociation symptom in PTSD[2] and the default mode network is related to introspective thinking[3]. The abnormal connectivity in these regions, therefore, might indicate abnormality of self-recognition in PTSD. VC subjects also showed

altered connectivity in the bilateral posterior insula. Although VC subjects showed no PTSD symptoms, combat experience might leave some effect on brain functional connectivity. These abnormal connectivities were normalized after the rtfMRI-nf training. As the effect was seen both for active and sham feedback groups, the training experience itself could have positive effect on PTSD. Active feedback group, however, showed a more pronounced training effect than sham group, which indicates that the neurofeedback helped to enhance the positive effect of the training.

References:

- [1] Shehzad Z, et al. (2014): A multivariate distance-based analytic framework for connectome-wide association studies. *Neuroimage*. 93 Pt 1:74-94.
- [2] Lanius RA, et al. (2006): A review of neuroimaging studies in PTSD: heterogeneity of response to symptom provocation. *Journal of Psychiatric Research*. 40:709-729.
- [3] Hamilton JP, et al. (2015): Depressive Rumination, the Default-Mode Network, and the Dark Matter of Clinical Neuroscience. *Biol Psychiatry*. 78(4):224-30.

C) EEG coherence enhancement during the rtfMRI-nf training in PTSD veterans

Introduction: We investigate EEG correlates of the rtfMRI-nf amygdala emotional training procedure by conducting analyses of EEG coherence, which is an EEG measure of functional connectivity. We hypothesized that EEG coherence during the rtfMRI-nf task would increase across the rtfMRI-nf runs. We observed that the enhancement in EEG coherence among the left fronto-temporal EEG channels significantly and positively correlated with the participants' PTSD severity ratings (CAPS).

Methods: The EEG coherence analysis was conducted for the EEG data, acquired during fMRI, after careful pre-processing and artifact removal using ICA. The EEG coherence was computed in Brain Vision Analyzer 2 as the ratio of cross-spectrum and auto-spectrum. The coherence values were averaged for an individual upper-alpha EEG band [IAF...IAF+2] Hz as in [1].

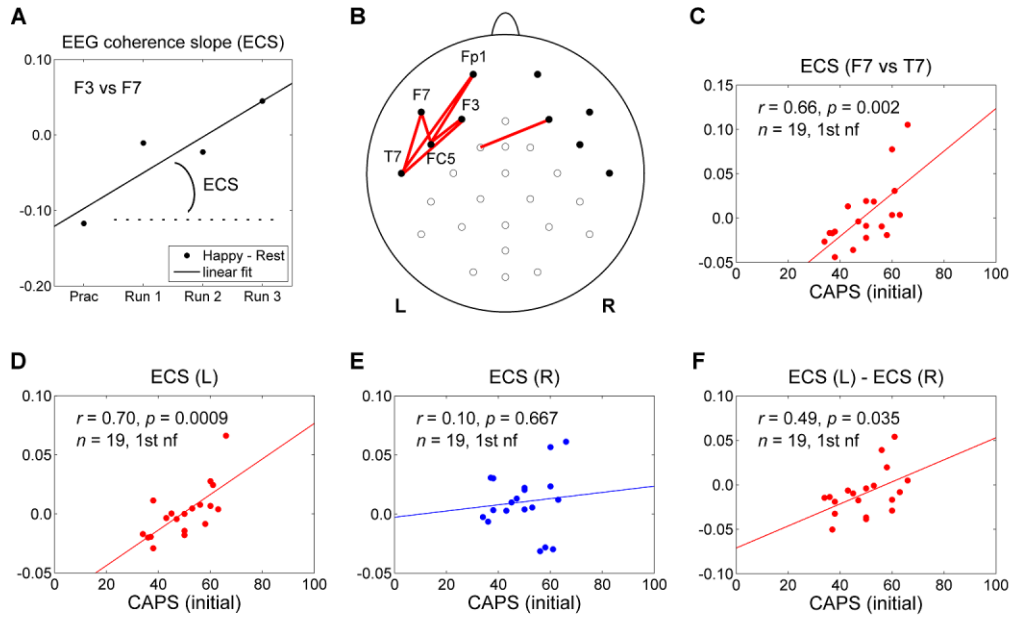


Figure C1. EEG coherence enhancement during the 1st rtfMRI-nf session. A) Definition of the EEG coherence slope (ECS) for a pair of EEG channels across four rtfMRI-nf runs. B) EEG channel pairs that exhibited positive correlations ($p < 0.01$, uncorr) between the ECS and the initial CAPS ratings. C) Illustration of such correlation for one channel pair. D) ECS(L) is the average ECS among fronto-temporal channels Fp1, F3, F7, FC5, T7 on the left. E) ECS(R) is the average ECS among the corresponding channels Fp2, F4, F8, FC6, T8 on the right. F) Correlation between the average ECS laterality and the initial CAPS ratings. See [1].

The EEG coherence slope (ECS) was defined as a slope of a linear trend in EEG coherence difference between the Happy and Rest conditions across the four rtfMRI-nf runs (Fig. C1A).

Results: The average ECS values for the left fronto-temporal EEG channel pairs during the 1st rtfMRI-nf session exhibited significant positive correlations with the initial CAPS ratings (Fig. C1B,C,D). Correlations for the right fronto-temporal EEG channel pairs were not significant (Fig. C1B,E). The average ECS laterality also significantly correlated with the CAPS ratings (Fig. C1F). Similar effects were observed for the 3rd rtfMRI-nf session: the average ECS values for the left fronto-temporal channel pairs showed significant correlations with the final CAPS (Fig. C2).

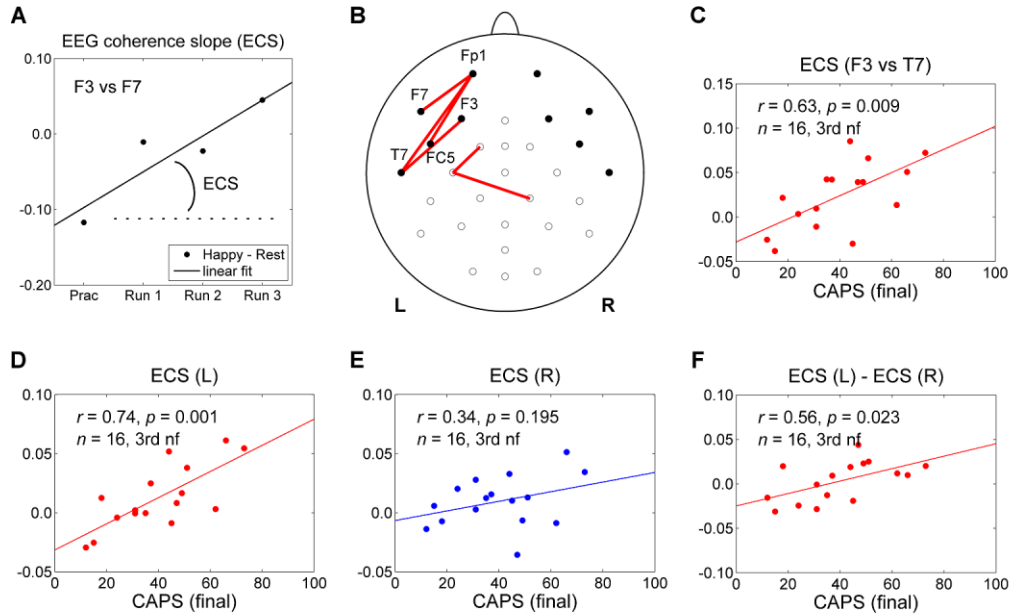


Figure 2. EEG coherence enhancement during the 3rd rtfMRI-nf session. Notations are the same as in Fig. 1. The ECS results are compared with the final CAPS ratings.

Discussion: Our results demonstrated that variations in EEG coherence during the rtfMRI-nf procedure are sensitive to severity of PTSD symptoms. The enhancement in EEG coherence among the left fronto-temporal EEG channels can be interpreted as an indication of enhancement in approach motivation [1]. The significant positive correlation between the ECS(L) and CAPS (Figs. C1D, C2D) suggests that the rtfMRI-nf procedure may be able to correct the approach motivation deficiencies specific to PTSD. The lack of significant inverse correlation between the ECS(R) and CAPS (Figs. C1E, C2E) suggests that the avoidance motivation might not be reduced. Nevertheless, the significant positive correlation between the average ECS laterality and CAPS (Figs. C1F, C2F) suggests that the overall motivational changes are positive and beneficial to PTSD patients. Similar results were previously observed for MDD patients [1].

References:

[1] Zotev V., et al, Correlation between amygdala BOLD activity and frontal EEG asymmetry during real-time fMRI neurofeedback training in patients with depression *NeuroImage: Clinical* 11 (2016) 224-238.

D) Correlation between amygdala BOLD activity and frontal EEG asymmetry in PTSD

Introduction: We investigate connection between frontal EEG asymmetry and BOLD activity during the rtfMRI-nf training in PTSD by performing EEG-fMRI correlation analysis. We hypothesized that temporal correlation between frontal EEG asymmetry and BOLD activity of the amygdala would be enhanced during the rtfMRI-nf task compared to a control task. Our analysis confirmed this hypothesis and provided new insights into functional deficiencies in PTSD.

Methods: The EEG-fMRI correlation analysis was performed as described in detail in [Ref1]. Frontal EEG asymmetry was defined as either $\ln(P(F4)) - \ln(P(F3))$ or $\ln(P(F8)) - \ln(P(F7))$, where P is EEG power in the upper alpha band. The time course of frontal EEG asymmetry was used to define two terms for the psychophysiological interaction (PPI) analysis: correlation and interaction. The [EEG-asymmetry-based regressor] \times [Happy-Count] interaction term described the difference in temporal correlations of the frontal EEG asymmetry (convolved with the HRF) and BOLD activity between the Happy and Count conditions. The PPI analysis was conducted within the GLM framework for all brain voxels [1].

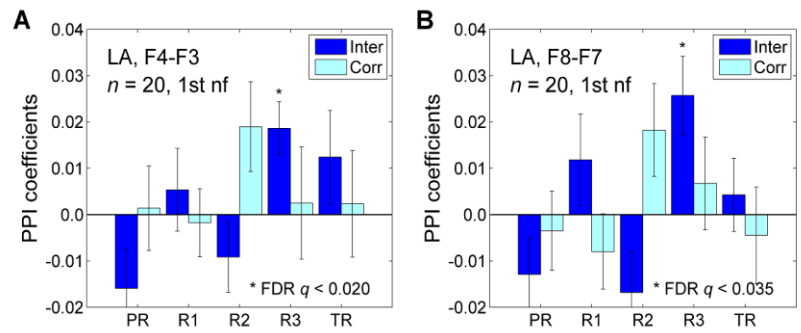


Figure D1. Enhancement in temporal correlation between frontal EEG asymmetry and BOLD activity of the left amygdala (LA) during the rtfMRI-nf training. A) Average PPI coefficients for the LA ROI corresponding to frontal EEG asymmetry $\ln(P(F4)) - \ln(P(F3))$. B) Average PPI coefficients corresponding to frontal EEG asymmetry

Results: The PPI interaction effect for the frontal EEG asymmetry $\ln(P(F4)) - \ln(P(F3))$, averaged within the LA ROI (Fig. D1A), was positive and significant for the last rtfMRI-nf run (R3) and exhibited a significant linear trend across the nf runs. This means that temporal correlation between the frontal EEG asymmetry and the LA BOLD activity was significantly enhanced during the Happy condition with rtfMRI-nf compared to the Count condition. Similar PPI effects were observed for the $\ln(P(F8)) - \ln(P(F7))$ asymmetry (Fig. D1B).

The whole-brain PPI interaction map (Fig. D2) is generally consistent with the PPI interaction map reported previously for MDD patients [1]. However, the PPI interaction effect is considerably weaker (Fig. D2) for the left lateral orbitofrontal cortex (LOFC) and the left rostral anterior cingulate cortex (rACC).

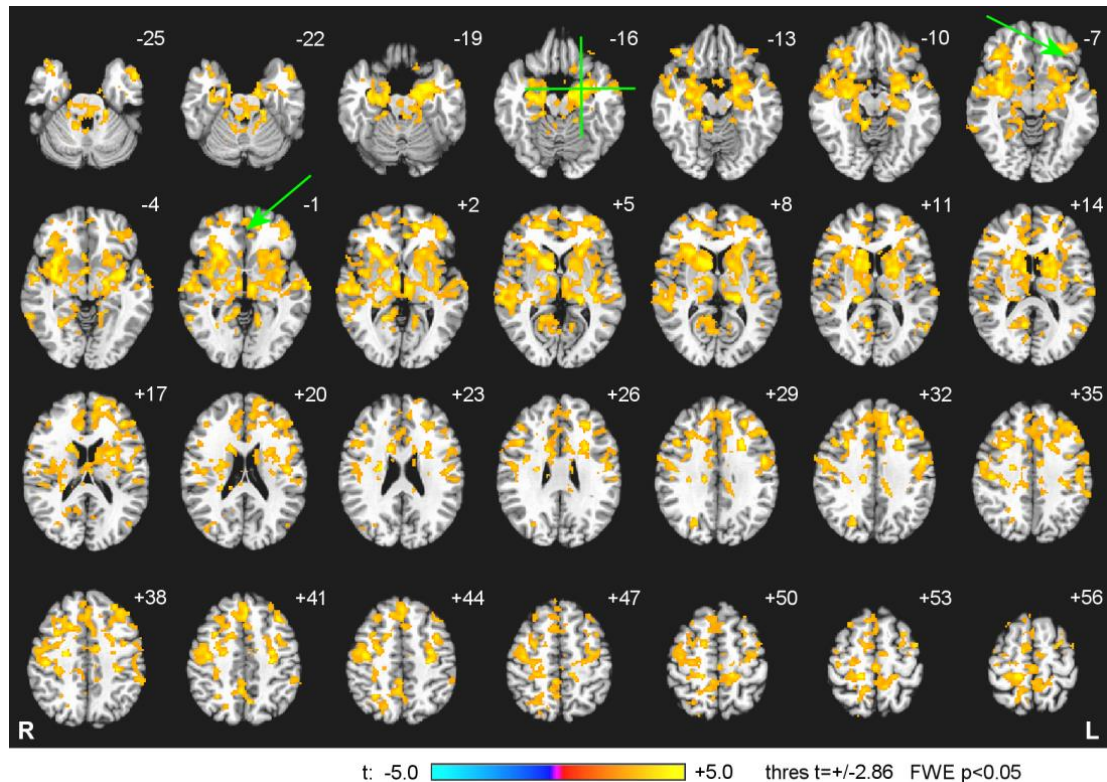


Figure D2. Group statistical map of the PPI interaction effect corresponding to frontal EEG asymmetry $\ln(P(F8)) - \ln(P(F7))$. The green crosshairs mark the center of the LA target ROI. The green arrows point to regions (LOFC and rACC) for which the PPI interaction effects in PTSD are substantially weaker than in MDD. See [1].

Discussion: Our results demonstrate that frontal EEG asymmetry provides relevant information about the participants' emotional/motivational states during the rtfMRI-nf training not only in MDD [1], but also in PTSD. Frontal EEG asymmetry can be used to indirectly probe activity of the amygdala and the related network by means of EEG. The weak PPI interaction effects for the LOFC and rACC (Fig. D2) can be attributed to the fact that activities of these regions are strongly affected by PTSD symptoms. This conclusion is supported by an independent (though not included here) fMRI functional connectivity analysis.

References:

[1] Zotev V., et al, Correlation between amygdala BOLD activity and frontal EEG asymmetry during real-time fMRI neurofeedback training in patients with depression *NeuroImage: Clinical* 11 (2016) 224-238.

E) Tracking Resting State Connectivity Dynamics in Veterans with PTSD

Introduction: In PTSD abnormal connectivity of spontaneous activity in several brain regions constituting the so-called default mode network (DMN) at resting state has been reported [1-3]. However, the mechanisms underlying these brain abnormalities are not fully resolved. Simultaneous electroencephalography (EEG) and BOLD fMRI have allowed for probing brain activity with joint high spatial and temporal resolution. Here, we acquired concurrent EEG and BOLD fMRI in groups of unmedicated veterans with combat-related PTSD and healthy veterans at rest and developed a novel multimodal analysis approach using temporal independent EEG microstates [4] to study DMN activity.

Methods: Simultaneous EEG and fMRI data were from 23 veterans with combat-related PTSD and 19 combat-expose veterans (combat-exposed controls, CEC) with eyes open in a resting state. BOLD fMRI RSNs were derived from preprocessed imaging data using spatial independent component analysis (ICA) separately for PTSD and CEC groups. The default mode network was selected by choosing the best-fit component with a template of the DMN [5]. The difference between groups was assessed using a two-sample unpaired t test. After correcting the MRI and cardioballistic artifacts, temporal independent EEG microstates (EEG-ms) were derived using the method described in [4]. We have identified temporal independent EEG-ms for each participant, and then obtained CEC and PTSD group results (Fig. E1). The DMN-related EEG-ms was selected by choosing one EEG-ms of correlated time course with BOLD fMRI DMN. The complete time courses of DMN-related EEG-ms were obtained by back-projection and determined via a winner-take-all approach. The occurrence frequency of DMN-related EEG-ms was calculated per each subject then compared across groups and against the clinical ratings.

Results: The demographic and clinical characteristics of all subjects are listed in Table E1.

Table E1. Participants demographics and clinical characteristics	PTSD (n=23)	CEC (n=19)
Age (Mean \pm SD Years)	34 \pm 9	32 \pm 7
PCL-M(Mean \pm SD) ***	43 \pm 15	18 \pm 3
CAPS(Mean \pm SD) ***	54 \pm 19	5 \pm 5
HARS (Mean \pm SD) ***	14 \pm 6	3 \pm 4
SHAPS(Mean \pm SD) ***	30 \pm 5	18 \pm 2
HDRS (Mean \pm SD) ***	14 \pm 6	3 \pm 4
MADRS (Mean \pm SD)***	17 \pm 8	2 \pm 4

*** significant difference between groups at $p < 0.001$

Figure E1 shows two sets of ten identified EEG microstates for both HC (upper row), and PTSD (lower row) groups. Nine out of the ten microstates highly resemble those found in our previous study in Yuan et al. 2012 [4].

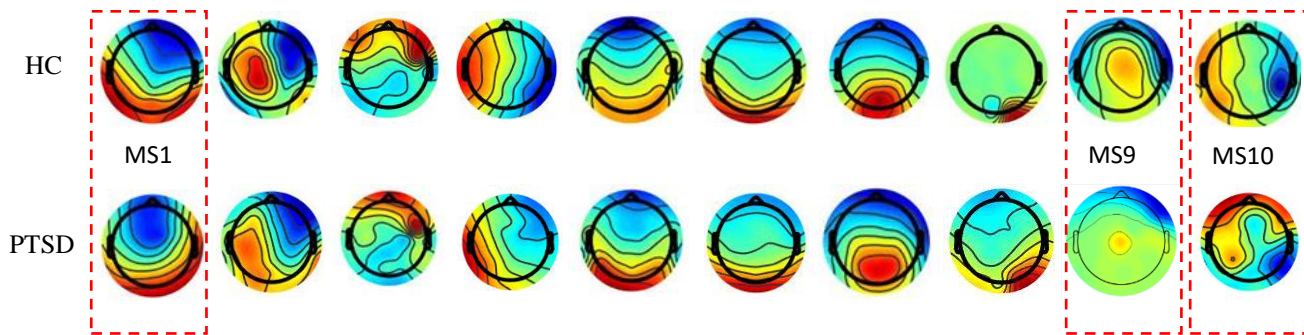


Fig. E1 EEG microstates identified in HC and PTSD groups. The pairs of microstates in dashed lines (MS1, M9, and MS10) show distinct features between HC and PTSD groups.

As the dynamics of the temporal independent microstates were reconstructed from EEG time series, it allows us to examine their signatures at a millisecond time scale. Among these EEG-ms, three microstates demonstrated distinctive differences in their fast evolving dynamics. EEG-ms that differ across both patient groups are marked by dashed lines in Fig. E1. The occurring frequencies of these three microstates are significantly different between HC and PTSD groups.

To further explore the neuronal substrates of these three signature microstates, the temporal dynamics of the microstates were compared with the time courses of BOLD signals after convolving with impulse hemodynamic response function. Regions where BOLD and EEG microstate time series are correlated were identified using a general linear model.

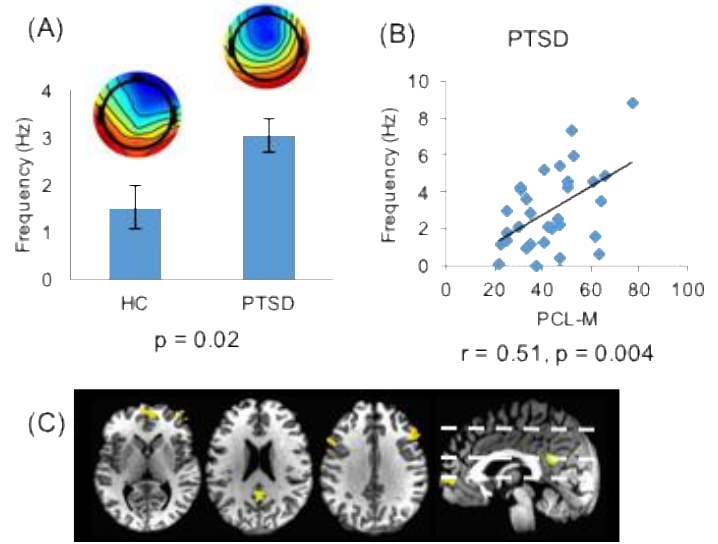


Fig. E2 EEG microstate MS1 that differ between HC and PTSD groups. (A) occurring frequency of microstate in HC and PTSD groups (B) occurring frequency vs. PCL-M scores in PTSD group (C) maps of correlation between BOLD and MS1 microstate time courses.

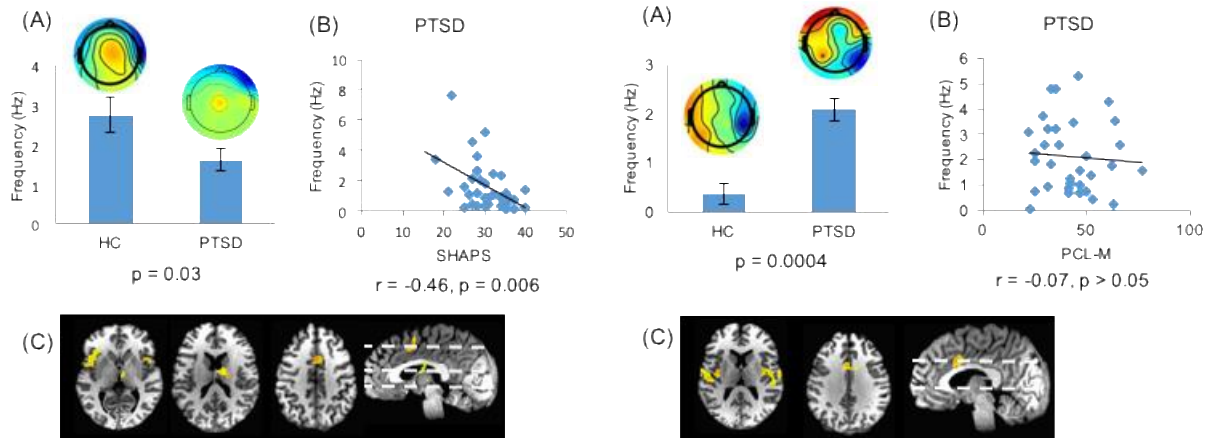


Fig. E3 EEG microstates (MS9, left panel, and MS10 right panel) that differ between HC and PTSD groups. (A) occurring frequency of microstate in HC and PTSD groups (B) MS9 occurring frequency vs. PCL-M scores (left panel) and MS10 occurrence ratio vs SHAPS (right panel) in PTSD group (C) maps of correlation between BOLD and microstate time courses.

The default model network (DMN) was identified therefore in correlation with one of the EEG microstates as shown in Figure E2. Importantly, the occurring frequency of the DMN-associated EEG microstates shows distinctive temporal dynamics between HC and PTSD groups (i.e., faster in PTSD subjects).

Moreover, the occurring frequency of such EEG microstates was also linearly related to the scores of PCL-M across the individuals in the PTSD group, indicating that more severe

symptom levels of PTSD are associated with faster dynamics of the DMN network. While functional MRI was able to pinpoint the anatomical regions of DMN, simultaneous EEG offers fast temporal dynamics that facilitate relating to the severity of symptoms. Two other microstates MS9 and MS10, were also found to be associated with distinctive dynamics between PTSD and HC groups (Fig E3, left and right panels respectively).

Discussion: From the simultaneously acquired EEG-fMRI data we identified temporal independent EEG microstates. Although EEG-ms temporal dynamics evolve at a much faster scale (order of milliseconds versus seconds for fMRI), we found an EEG-ms that was correlated with the fMRI DMN network (MS1) and MS9, and MS10 interestingly identified a similar insular network including bilateral insula, the cingulate cortex and the medial temporal cortex. However, the temporal dynamics of MS9 and MS10 (Fig. E3, left and right panels respectively) show dramatically different characteristics. For MS10, PTSD subjects showed significantly higher occurring frequency than the control veterans, whereas for microstate MS9, PTSD subjects showed lower occurring frequency. Furthermore, the dynamics of MS10 did not show any significant linear trend between the occurring frequency and the level of symptoms ($p > 0.05$ for both PCL-M and SHAPS). On the contrary, the dynamics of MS9 was found to negatively correlate with SHAPS scores across the individual subjects. The occurrence frequency of MS1 EEG-ms (Fig. E2) statistically differentiates between PTSD and HC group. Importantly, this EEG-ms occurrence frequency positively correlated with PTSD symptom severity (PCL-M). The abnormally decreased functional connectivity in PTSD veterans observed by fMRI was associated with decreased occurrence frequency of DMN-related EEG-ms, which suggests dynamic relocation of neural processing resources associated with the PTSD condition.

References:

- [1] Rauch, S. L. (2006), Neurocircuitry models of posttraumatic stress disorder and extinction: human neuroimaging research--past, present, and future. *Biol Psychiatry*, vol. 60, pp. 376-82.
- [2] Bruce, S.E., (2012), Altered emotional interference processing in the amygdala and insula in women with Post-Traumatic Stress Disorder *Neuroimage Clin*, vol. 2, pp. 43-49.
- [3] Yan, X., (2014), *Psychiatry Res*, pii: S0925-4927(14)00328-X.
- [4] Yuan, H. (2012), Spatiotemporal dynamics of the brain at rest – exploring EEG microstates as electrophysiological signatures of BOLD resting state networks *Neuroimage*, vol. 60, no. 4, pp. 2062-2072.
- [5] Laird, A.R. Investigating the functional heterogeneity of the default mode network using coordinate-based meta-analytic modeling (2009), *J Neurosci*, vol. 29, no. 46, pp. 14496-14505.

Summary of preliminary data analyses

Taken together, preliminary analyses of our rtfMRI-nf amygdala training results demonstrate feasibility of the rtfMRI-nf amygdala training procedure. All study participants tolerate this procedure well. We have observed large individual variability in learning ability to control left amygdala hemodynamic activity during rtfMRI-nf procedure. However, data suggests that veterans with PTSD can learn to self-regulate their amygdala BOLD responses during recall of positive autobiographical memories (i.e., confirmation of Aim #1). Notably, rtfMRI-nf training of the left amygdala resulted in statistically and clinical significant improvements in PTSD (CAPS, PCL-M) and depression symptoms (HRSD). Significant reduction in PTSD and depression symptoms were seen in veterans with PTSD in the experimental group but not in controls.

Functional connectivity analysis of the amygdala during the neurofeedback procedures revealed substantial differences between the experimental (feedback from LA) and control groups (feedback from HIPS), proving additional evidence of a specific neuromodulatory effect induced by the LA neurofeedback procedure during positive memory recall. Brain regions co-activated with the LA feedback procedure (forming an amygdala-related network) were consistent with the broader literature regarding the amygdala-related neural network involved in emotion processing. We found that veterans with PTSD had higher connectivity across motor areas, which could associate with hyperarousal symptom. In addition, we found hyperconnectivity between the right superior temporal region and default mode network regions, which could associate with dissociation symptom in PTSD, indicating abnormalities in self-referential in PTSD. Interestingly, we also found in trauma-exposed veterans without PTSD diagnosis altered connectivity in bilateral posterior insula, suggesting that military training and/or combat experience might affect brain connectivity. Notably, these abnormal connectivities were normalized after rtfMRI-nf amygdala training, although we did not see statistically significant difference between active and control/sham condition. Lack of statistically significant difference between active and control/sham condition is likely due to large individual subject variability, exclusion due to head motions, and simply the use of all available PTSD subjects without any classification (i.e. responders vs non-responders to rtfMRI-nf procedure, our currently ongoing classification efforts). Nevertheless, the active feedback

group showed more pronounced training effect than control/sham group, which indicates that the rtfMRI-nf amygdala emotional training is beneficial to veterans with PTSD.

Ongoing analysis of the concurrently acquired EEG data during rtfMRI-nf revealed that modulation of BOLD LA activity during the neurofeedback procedure was accompanied by changes in frontal EEG asymmetry (FEA) in the upper alpha band (power(F4)-power(F3) electrodes). The direction of change in the FEA (e.g., more positive FEA) induced by the rtfMRI-nf LA training was consistent with more approach-oriented responses and traits as well as more positive emotions. Indeed, we observed that reduction in CAPS ratings was associated with reduction in the average FEA changes during the rtfMRI-nf task, indicating that variations in FEA during rtfMRI-nf training might independently provide valuable information about PTSD severity and treatment response. Those preliminary results identified the FEA as a promising target for currently ongoing EEG-only neurofeedback training among veterans with combat-related PTSD (Aim #3).

During the rtfMRI-nf training of the amygdala, EEG coherence among left fronto-temporal EEG channels in the upper alpha band shows enhancement that significantly correlates with PTSD severity. Findings demonstrate that variations in EEG coherence during the rtfMRI-nf procedure are sensitive to severity of PTSD symptoms. Notably, such left-lateralized EEG coherence enhancement, indicating increased coherent neuronal activity, suggests an increase in approach motivation, which is more pronounced in patients with more severe PTSD. No reduction in EEG coherence among right fronto-temporal EEG channels is observed, suggesting that the employed rtfMRI-nf procedure does not lead to a significant reduction in avoidance motivation in PTSD. Nevertheless, the significant positive correlation between the average EEG coherence slope laterality and CAPS suggests that the overall motivational changes are positive and beneficial to PTSD patients. Similar results were previously observed for MDD patients [30].

We have identified temporal independent EEG microstates in individuals with combat related PTSD and found ones that differs between people with PTSD and healthy controls. The occurrence frequency of the EEG-ms which was associated with BOLD DMN, statistically differentiates PTSD and HC groups and positively correlates with PTSD symptom severity.

Recruitment efforts description

During the fourth year of the grant (October 2015 through September 2016) radio advertising continued to be our most successful recruitment effort. We ran 15 ads for a total of 1221 ad spots on 4 different radio stations across multiple genres (country, rock, hip-hop, and contemporary hits) in the Tulsa area. Radio stations were chosen based on previous success as well as Nielson ratings. Due to the large number of calls we got when running ads on and around Independence Day we increased the number of advertisements airing during the week of Veterans Day this year. For one ad run we also were the sponsors of the live read traffic for the morning and evening commute. In addition to advertisements, LIBR staff members participated in monthly on-air interviews the hip-hop station, which is the highest rated station for minority listeners in the Tulsa area according to Arbitron.

This year we also aired a television ad for the study. The advertisement aired on the local FOX station as well as the local MyNetworkTV station. There were a total of 290 ad spots over 19 days in June. Air times were selected based on TV ratings for male viewers age 18 to 55. In addition to these media broadcast advertisements, the study was featured on a local news program in June. The segment was 3 minutes long and aired during the 5 o'clock news on the Tulsa NBC channel. It featured a veteran who had completed phase 1 of the study and showed the rtfMRI-nf with simultaneous EEG procedure.

We have also continued running advertisements on Facebook on a quarterly basis. These advertisements reach up to 200,000 individual accounts and target both male and female users to maximize the number of both direct and indirect referrals. In addition to these study-specific ads, general advertisements for LIBR on radio, electronic media, and bus stations also refer veterans to our study.

We have also continued our community outreach efforts during the last year. LIBR staff participated in the PURPOSE 2.2 Run/Walk, an event to raise awareness of veteran suicide, on April 30, 2016. LIBR also promoted the study at a number of events throughout the year, including the North Tulsa Neighborhood Summit (August 27, 2016) and the Zarrow Symposium (September 28-30, 2016). We have an on-going monthly relationship with certain medical and veteran organizations such as the Veterans Initiative, Family and Children Services, Veteran's Advisory Council, and Laureate Psychiatric Clinic and Hospital. Each month, our recruitment-focused staff attended meetings and provided presentations provided study-focused literature to

various social workers, mental health counselors, psychologists, and physicians. These recruitment efforts included meetings and presentations with selected clinicians who work directly with the target population. We also continued our efforts directly targeting recruiting patients/potential participants from Family and Children Services monthly and the Laureate Psychiatric Clinic and Hospital weekly.

As we are now recruiting exclusively for phase 3 of the study, we have rewritten some of our advertising material to align with the participation criteria for the EEG-only experiments. We hope that rerecording our radio advertisements may also have the effect of hooking listeners who grew accustomed to hearing the old ad. Additionally, we went through our database of subjects who had been excluded from the first phase of the study due to metal in the body or size restrictions and contacted them about participating in phase 3.

Enrollment information

Between October 1, 2015 and September 30, 2016:

- 414 veterans contacted LIBR
- 367 veterans completed phone screens
- 114 completed initial screening
 - 34 found eligible for the current study
- 10 participants signed the consent for the study
- 4 participants completed the study

Phase 3:

- 8 consented to the study
- 6 completed the 1st scan
- 4 completed the 2nd scan
- 4 completed the 3rd scan
- 4 completed the 4th scan
- 4 completed the final scan
- 4 completed the final CAPS

Phase 1:

- 71 consented to the study
- 62 completed the 1st scan
- 57 completed the 2nd scan

- 52 completed the 3rd scan
- 49 completed the 4th scan
- 47 completed the final scan
- 37 completed the final CAPS

In the upcoming months, recruitment will continue to research new ways to help recruit the target population. Radio ads have consistently been our most effective recruitment method, so we will continue to run regular ads on the radio. We already have a number of recruitment events scheduled for the next year, including the Tulsa NAMI Conference and meeting with the Laureate Psychiatric Clinic and Hospital intensive outpatient groups.

- 4. KEY RESEARCH ACCOMPLISHMENTS:** Bulleted list of key research accomplishments emanating from this research. Project milestones, such as simply completing proposed experiments, are not acceptable as key research accomplishments. Key research accomplishments are those that have contributed to the major goals and objectives and that have potential impact on the research field.

Due to recruitments delays and continuing EEG data collection, there is nothing to report for the period covered by this report.

- 5. CONCLUSION:** Summarize the importance and/or implications with respect to medical and /or military significance of the completed research including distinctive contributions, innovations, or changes in practice or behavior that has come about as a result of the project. A brief description of future plans to accomplish the goals and objectives shall also be included.

Recruitment of veterans continued to be challenging which has resulted in data collection delays and in our request on August 2, 2016 for a second 12-month no-cost extension, which was approved (beyond the original project complete date of Sep 29, 2015). During the last year we have improved our recruitment. We are continuing to secure even more institutional support for our already very substantial recruitment efforts to further increase our recruitment campaign focused on the veteran population in Oklahoma. Those substantial efforts (as described in this report) improved subject enrollment rate. We anticipate that those efforts will be sufficient and realistic to accomplish our pending aims of this project.

Our other efforts during year 4 have resulted in substantial progress toward accomplishing our aims for this project. We have completed the rtfMRI-nf and EEG experimental Phase 1 of the study and advanced necessary methodological aspects of the study, including development and implementation of data analysis pipelines and conducting data analysis on collected multimodal fMRI and EEG data. All study participants tolerate the rtfMRI-nf emotional training well. Despite large individual subject variability in controlling amygdala hemodynamic activity during the rtfMRI-nf, ongoing data analysis suggests rtfMRI-nf feasibility and clinical relevance in reducing PTSD symptoms. For the purpose of accomplishing Aim #2, we have developed and continue to improve a software environment for real-time EEG neurofeedback (rtEEG-nf). Feasibility of rtfMRI-nf amygdala training (with simultaneous EEG recordings) in the combat-related PTSD population (Aim #1) allowed for identification of an EEG signal feature (frontal EEG asymmetry) for the purpose of establishing rtEEG neurofeedback. We have developed and implemented an EEG-nf training paradigm (Aim #3 and Phase 3), and data collection is currently underway. We anticipate that our accomplishments in Years 1 through 4 have situated our successfully collaborating team for further satisfactory progress throughout the remainder of the project period. Therefore, we remain well-positioned to develop and proof-of-concept a novel intervention that has the potential to advance both understanding of PTSD and our ability to more successfully treat this chronic and costly condition.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

- a. List all manuscripts submitted for publication during the period covered by this report resulting from this project. Include those in the categories of lay press, peer-reviewed scientific journals, invited articles, and abstracts. Each entry shall include the author(s), article title, journal name, book title, editors(s), publisher, volume number, page number(s), date, DOI, PMID, and/or ISBN.

- (1) Lay Press:

- (2) Peer-Reviewed Scientific Journals:

- (3) Invited Articles:

- (4) Abstracts:

- b. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

a. Submitted manuscripts:

Peer-Reviewed Scientific Journals:

- 1) Wong CK, Zotev V, Misaki M, Phillips R, Luo Q, Bodurka J. "Automatic EEG-assisted retrospective motion correction for fMRI (aE-REMCOR)." *Neuroimage*. 2016 Apr 1;129:133-47. doi: [10.1016/j.neuroimage.2016.01.042](https://doi.org/10.1016/j.neuroimage.2016.01.042).
- 2) Mayeli A, Zotev V, Refai H, Bodurka J. "Real-time EEG artifact correction during fMRI using ICA." *J Neurosci Methods*. 2016 Sep 30;274:27-37. doi: 10.1016/j.jneumeth.2016.09.012.

b. Conference abstracts:

- 1) Zotev, V., Phillips, R., Misaki, M., Wong, C.K., Wurfel, B., Meyer, M., Krueger, F., Feldner, M., Bodurka, J. (2015). Evaluation of rtfMRI Neurofeedback Training Effects in Combat-related PTSD Using Simultaneous EEG. E-poster presented at the 24th Annual Meeting of the International Society of Magnetic Resonance in Medicine, Singapore. In: *Proc. Intl. Soc. Magn. Reson. Med.* 24, 280
- 3) *Mayeli, A., Zotev, V., Refai, H., Bodurka, J. (2016). Real-time ICA-based artifact correction of EEG data recorded during functional MRI. E-poster presented at the 24th Annual Meeting of the International Society of Magnetic Resonance in Medicine, Singapore. In: *Proc. Intl. Soc. Magn. Reson. Med.* 24, 3773
- 3) Wong, C.K., Zotev, V., Misaki, M., Phillips, R., Luo, Q., Bodurka, J. (2016). Automatic EEG-assisted retrospective head motion correction improves rs-fMRI connectivity analysis. Poster presented at the 24th Annual Meeting of the International Society of Magnetic Resonance in Medicine, Singapore. In: *Proc. Intl. Soc. Magn. Reson. Med.* 24, 3774
- 4) Wong, C.K., Zotev, V., Misaki, M., Phillips, R., Luo, Q., Bodurka, J. (2016). Support vector machine classification of head motion independent components from EEG-fMRI. Poster presented at the 22nd Annual Meeting of the Organization for Human Brain Mapping, Geneva, Switzerland. In: *Conf. Proc.* 1811.

5) Phillips, R., Zotev, V., Young, K., Misaki, M., Wong, C.K., Wurfel, B., Meyer, M., Krueger, F., Feldner, M., Bodurka, J. (2016). Changes in Amygdala Connectivity During Multiple Visits of Real-Time fMRI Neurofeedback Training. Poster presented at the 22nd Annual Meeting of the Organization for Human Brain Mapping, Geneva, Switzerland. In: Conf. Proc. 3951.

c. Presentations:

- 1) Bodurka, J. (2015). Emotional regulation training of amygdala using real-time fMRI and EEG-assisted neurofeedback in combat-related PTSD. Talk (seminar) at the 31st Annual Meeting of the International Society for Traumatic Stress Studies, New Orleans, Louisiana, USA.
- 2) Bodurka, J. (2015). Emotional regulation training with real-time fMRI neurofeedback of the amygdala and simultaneous EEG measurements. Talk (key note) at the Neuroscience Symposium, Central Institute for Mental Health, Mannheim, Germany.
- 3) Bodurka, J. (2016). Emotion Regulation Training with Real-Time fMRI Neurofeedback of the Amygdala and Concurrent EEG Recordings in Combat-Related PTSD. Talk at the 13th Annual Meeting of the Society for Brain Mapping and Therapeutics, Miami, Florida, USA
- 4) Bodurka, J. (2016). Advances in multimodal MRI and EEG Imaging for studying the human brain. Talk (invited first inaugural speaker of the Stephenson School of Biomedical Engineering seminar series), Oklahoma University, Norman, Oklahoma, USA.
- 5) Bodurka J. (2016). Emotion Regulation Training of Amygdala using Real-time fMRI and EEG-assisted Neurofeedback. Talk (seminar) at the 22nd Annual Meeting of Organization for Human Brain Mapping, Geneva, Switzerland.

- 6. INVENTIONS, PATENTS AND LICENSES:** List all inventions made and patents and licenses applied for and/or issued. Each entry shall include the inventor(s), invention title, patent application number, filing date, patent number if issued, patent issued date, national, or international.

Nothing to report.

- 7. REPORTABLE OUTCOMES:** Provide a list of reportable outcomes that have resulted from this research. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. This list may include development of prototypes, computer programs and/or software (such as databases and animal models, etc.) or similar products that may be commercialized.

- 1) We have completed development of an enhanced an automated implementation of EEG-assisted retrospective motion correction (E-REMCOR), which utilizes EEG motion artifacts to correct the effects of head movements in simultaneously acquired fMRI data on a slice-by-slice basis [27]. The automated implementation of E-REMCOR, referred to as aE-REMCOR [28], was developed to facilitate the application of E-REMCOR in multimodal brain research and in particular in large-scale clinical EEG-fMRI studies. The aE-REMCOR algorithm, implemented in MATLAB, enables automated preprocessing of the EEG data, ICA decomposition, and, importantly, automatic, computer-based identification of motion-related ICs. The aE-REMCOR is capable of substantially reducing head motion artifacts in fMRI data. We found that veterans with PTSD tend to move more during fMRI scans as compared to other patient groups and healthy controls. Therefore, we have continued to apply the method on all acquired fMRI and EEG data from veterans with combat-related PTSD.
- 2) We are continuing development of better algorithms for artifact suppression in EEG data acquired simultaneously with fMRI. Specifically, we have developed an improved method for automatic period detection of cardioballistic artifacts in EEG-fMRI data. Because of the presence of high magnetic field in fMRI, inaccurate heart beat period detection using the electrocardiogram (ECG) recording is often observed. Since the waveform of the BCG artifact in EEG-fMRI data is relatively invariable as compared to the ECG waveforms, we

propose a multiple-scale peak detection algorithm to determine directly the BCG period. The proposed algorithm achieves a higher and better detection accuracy of the artifact occurrence on a large EEG dataset in EEG-fMRI, and importantly without using the ECG recordings. It virtually eliminates the need of ECG for BCG artifact removal. Importantly, it can be applied retrospectively on the large EEG-fMRI data sets already acquired.

- 3) Abnormal brain resting state connectivity dynamics in PTSD: novel insights from simultaneous EEG and fMRI. We have completed, on a larger number of unmedicated veterans with combat-related PTSD and trauma-exposed healthy veterans, an exploratory multimodal analysis on the EEG-fMRI data collected during resting scans. This novel analysis approach uses temporal independent EEG microstates (EEG-ms) [16] to study default mode network activity (DMN) activity. From the simultaneously acquired EEG-fMRI data we identified temporal independent EEG microstates whose temporal dynamics evolve at much faster scale and yet we found EEG-ms (MS1) that is correlated with the fMRI DMN network, and found another EEG-ms that correlates with insular network including bilateral insular, the cingulate cortex and the medial temporal cortex. The occurrence ratio of the EEG-ms MS1, MS9, and MS10 statistically differentiates between PTSD and HC group. Additionally, the MS1 occurrence ratio positively correlates with PCL-M scores, and MS9 occurrence ratio negatively correlates with SHAPS scores in veterans with PTSD. Importantly, the abnormally decreased functional connectivity in veterans with PTSD observed via fMRI was associated with a decreased occurrence ratio of DMN-related EEG-ms MS1, which suggests the relocation of neural processing resources associated with the PTSD condition. The ability to capture and measure brain connectivity dynamics with simultaneous EEG&fMRI and EEG-ms analysis provides very promising approach to discover endophenotypes in PTSD.

8. **OTHER ACHIEVEMENTS:** This list may include degrees obtained that are supported by this award, development of cell lines, tissue or serum repositories, funding applied for based on work supported by this award, and employment or research opportunities applied for and/or received based on experience/training supported by this award.

Nothing to report.

9. REFERENCES: List all references pertinent to the report using a standard journal format (i.e., format used in *Science*, *Military Medicine*, etc.).

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10. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

NOTE:

TRAINING OR FELLOWSHIP AWARDS: For training or fellowship awards, in addition to the elements outlined above, include a brief description of opportunities for training and professional development. Training activities may include, for example, courses or one-on-one work with a mentor. Professional development activities may include workshops, conferences, seminars, and study groups.

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.

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1) Zotev, V., Yuan, H., Misaki, M., Phillips, R., Young, K.D., Feldner, M.T. Bodurka J. Correlation between amygdala BOLD activity and frontal EEG asymmetry during real-time fMRI neurofeedback training in patients with depression. *NeuroImage Clinical* 2016 11:224-238
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